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Biological invasions have been argued to be facilitated by phenotypic plasticity. Although experiments have shown plasticity can be adaptive, our current understanding is limited to comparative studies that measure plasticity between invasive and non-invasive species or populations. Because plasticity in a trait is a property of a genotype, a more effective test would measure the fitness effects of genotypes that vary in plasticity. I used this approach to conduct an experiment using native European *Plantago lanceolata* genotypes that expressed plasticity in multiple reproductive traits. I transplanted these genotypes into two novel environments that differ in thermal regime during the reproductive season and measured the fitness effects of plasticity and source latitude. Results were consistent with the hypothesis that plasticity improves reproductive success in novel environments. However, the fitness effects of plasticity varied by trait, and environment. Phenotypic-selection analyses showed evidence of stabilizing selection for onset plasticity and directional selection favoring plasticity in stalk length and spike length. Results also provided evidence that source latitude and plasticity affect reproductive success independently of each other and that the effects differ between reproductive years. Phenotypic-selection analyses showed evidence that as source latitude increased, reproductive success increased in the short and cool environment but only in 2012. My results suggest that the role of plasticity should be examined in multiple vegetative and reproductive traits.

THE ROLE OF PHENOTYPIC PLASTICITY IN THE COLONIZATION OF NOVEL ENVIRONMENTS

by

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Approved by

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Committee Chair

To my supportive parents, Fredy, Janneth, and my little brother Kevin.

APPROVAL PAGE

This thesis written by Freddy O. Herrera has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Phenotypic plasticity has been widely cited as facilitating the successful establishment of species in novel environments (e.g. Dybdahl and Kane 2005; Richards et al 2006; Funk 2008; Hulme 2008). Because plasticity allows a genotype to express multiple phenotypes under different environmental conditions (Bradshaw 1965), it makes intuitive sense to assume that an organism with high plasticity can become established more easily than a low plasticity organism in novel environments that have different environmental conditions from those in which the organisms evolved (Richards et al 2006; Ghalambor et al 2007). Indeed, this hypothesis was first proposed by Baker (1965) who observed plasticity as the major difference between weedy and non-weedy species when these were grown in the greenhouse.

Plasticity is an inherent attribute of a trait for a genotype and can be subject to natural selection and, thus, can evolve (Bradshaw 1965). Phenotypic plasticity can be neutral, maladaptive or adaptive. Plasticity provides a mechanism for adaptation in spatially and temporally variable environments (DeWitt and Scheiner 2004). Plasticity has been argued to be adaptive when: 1) environments vary predictably, 2) natural selection favors a different phenotype in each environment, 3) the environmental cues that result in an induced phenotype are reliable, 4) the induced phenotype provides a

fitness advantage to the organism, and 5) no phenotype excels across all environments (Bradshaw 1965).

Evaluating whether or not plasticity is adaptive in nature requires the testing of plastic phenotypes and non-plastic phenotypes in each environment being considered. A plastic phenotype is only adaptive when it increases fitness relative to the non-plastic phenotypes in that environment. For example, in spatially variable environments, experiments have examined the effects of stem elongation plasticity on fitness. Fitness was greater for plastic phenotypes over non-plastic phenotypes only in high-density treatments (Dudley and Schmitt 1996; Schmitt el at 1999). Other experiments have shown that plasticity increases fitness in temporally variable environments. Different melanin concentrations, which facilitate thermoregulation, in Western white (*Pontia occidentalis*) butterflies affected survival differently during the reproductive season. Phenotypes with low-melanin concentration survived better but only later in the reproductive season (Kingsolver and Huey 1998). Floral reflectance plasticity, which allows for thermoregulation, in English plantain (*Plantago lanceolata*) affected reproductive success differently during the reproductive season. Highly plastic genotypes produced more seeds in the beginning of the reproductive season. This pattern switched later in the reproductive season when low plasticity genotypes produced more seeds. At the end of the reproductive season, genotypes with intermediate plasticity had the greatest reproductive success (Lacey et al 2012). Although these examples highlight the fact that plasticity can be adaptive under some environmental conditions, research linking successful establishment of novel environments due to plasticity is limited.

Several approaches have been implemented to test the hypothesis that high levels of plasticity have enabled species to colonize and establish in novel environments. Experiments have compared the plasticities of introduced invasive species to native species when plants were grown in greenhouses or common gardens (Schweitzer and Larson 1999; Jakobs et al 2004; Brock et al 2004; Funk 2008; Godoy et al 2011). Generally, these studies found evidence that the introduced invasive species had greater plasticity or that both groups displayed similar levels of plasticity. Experiments have also compared the plasticities of introduced invasive species from Europe or Asia to introduced non-invasive North American congeners when these were grown in greenhouses or common gardens. Burns (2004) found evidence that introduced invasive species had greater plasticity, whereas Muth and Pigliucci (2006) found no evidence of a relationship between invasiveness and plasticity. Lambrecht-McDowell and Radosevich (2005) and Flory et al (2011) compared the plasticity of introduced invasive populations from Europe and China to native populations from North America and China when these were grown in common gardens, respectively. In both experiments, there was no evidence that introduced populations expressed greater plasticity than native populations. Collectively, these studies provide ambiguous information on the role of plasticity in facilitating the establishment of invasive species in novel environments.

In order to understand the role, if any, of plasticity, a more direct approach would be to ask, "Do plastic and non-plastic genotypes collected from the native range of a species differ in fitness when they are introduced to new environments?" I am aware of no studies that have addressed this question. Therefore, I have used this approach to

evaluate the fitness effects of plasticity in *Plantago lanceolata* L. (Plantaginaceae). *P. lanceolata* is an invasive herb from Eurasia that has successfully colonized North America.

I focused on the plasticity of reproductive traits because reproduction is a major component of colonization ability in a new environment. Experiments have often measured plasticity in vegetative traits (e.g. Schweitzer and Larson 1999; Jakobs et al 2004), and few have investigated the plasticity of reproductive traits (Vogler et al 1999; Mal and Lovett-Doust 2005; Lacey and Herr 2005). Vogler et al (1999) observed that numerous reproductive traits like flower number, corolla size, and ovule number were plastic in the creeping bellflower (*Campanula rapunculoides*). Mal and Lovett-Doust (2005) observed that inflorescence mass was plastic in purple loosestrife (*Lythrum salicaria*), whereas Lacey and Herr (2005) observed that floral reflectance in English plantain (*Plantago lanceolata*) is plastic. To the best of my knowledge, only two experiments have directly examined the effects of plasticity in reproductive traits (Yeh and Price 2004; Lacey et al 2012). Yeh and Price (2004) observed dark-eyed Junco (*Junco hyemalis*) populations and found that plasticity in breeding onset resulted in more chick fledglings. Lacey et al (2012) used English plantain (*P. lanceolata*) in common garden experiments and found that floral reflectance plasticity affected reproductive success.

There is evidence that multiple reproductive traits in *P. lanceolata* are temperature-sensitive (Lacey and Herr 2005; Blank and Lacey unpublished). This response to temperature, which results in a genotype altering its phenotype, is called

thermal plasticity. Temperature-sensitive phenotypic plasticity might help to buffer the effects of climate change, which models predict to increase temperatures between 2°C and 7°C (IPCC 2007), and facilitate the colonization of novel environments through deliberate or accidental introductions by improving fitness. Therefore, to test the role of plasticity in the colonization of novel environments, I transplanted native European *P. lanceolata* genotypes into two different thermal environments found within the introduced range in North America. These sites differed in temperature and length of the reproductive season. I tested the hypothesis that plasticity in reproductive traits improves reproductive success in new environments. The hypothesis predicts that *P. lanceolata* plants that express more plasticity will produce more spikes and seeds during the reproductive season.

Reproductive biology of *Plantago lanceolata*

Plantago lanceolata L. (English plantain) is a weedy perennial herb native to Eurasia. It was introduced to North America around the 16th century and has widely spread growing in disturbed areas, lawns, roadsides, and grasslands (Sagar and Harper 1964; Cavers et al 1980). *P. lanceolata* grows vegetatively as a basal rosette. Flowering is photoperiodically controlled and starts with the production of long leafless-stalks. Each stalk gives rise to an inflorescence called a spike. A spike holds many inconspicuous flowers. A genotype can produce numerous spikes throughout the reproductive season (Lacey and Herr 2005; Lacey et al 2012). This process is similar to the production of multiple clutches in animals. Protogynous flowers are self-incompatible, are

predominately wind-pollinated, and persist until seed set at which time the spike and capsules turn brown.

Temperature-sensitive reflectance plasticity in *Plantago lanceolata*

Large temperature-induced changes in floral reflectance of *P. lanceolata* have been observed in the visible (400-700nm) and near-infrared (750-850nm) portion of the wavelength spectrum (Lacey and Herr 2005). At warm temperatures, all genotypes generally develop highly reflective and lightly colored flowers/spikes. At cool temperatures genotypes vary in their response. Low plasticity genotypes, which are temperature-insensitive, continue to produce highly reflective and lightly colored flowers/spikes. High plasticity genotypes, which are temperature-sensitive, produce poorly reflective and darkly colored flowers/spikes. Floral reflectance of most genotypes lies within these two extremes at cool temperatures. Floral reflectance plasticity allows a genotype to partially thermoregulate its reproduction by producing poorly reflective and darkly colored flowers/spikes in the spring, when its cool, and highly reflective and lightly colored flowers/spikes in the summer, when its warm (Lacey and Herr 2005; Lacey et al 2012). Thermoregulation allows a genotype to respond to external temperatures and maintain internal body temperatures closer to optimum.

Plasticity of other reproductive traits in *Plantago lanceolata*

There is some evidence that other reproductive traits are also plastic in *P. lanceolata*. A preliminary growth chamber experiment showed that flowering time, stalk length, and spike length in *P. lanceolata* are temperature-sensitive (Blank and Lacey unpubl.). Genotypes induced to flower under cooler temperatures began flower

production earlier and produced smaller stalks and spikes. Currently, we have no information on whether these and/or other reproductive traits in *P. lanceolata* express plasticity in nature.

Plasticity in several reproductive traits might theoretically contribute to *P. lanceolata* genotypes being able to partially thermoregulate their reproduction. Plasticity in flowering onset and in duration might allow a genotype to alter the timing of its reproductive season so that flowering begins under favorable environmental temperatures. Plasticity in the reproductive rate might allow a genotype to alter its reproduction so that peak flowering and seed development occur under favorable temperatures. Stalk length plasticity might allow a genotype to partially thermoregulate its reproduction by adjusting the distance of reproductive tissues to a heat source. For example, a genotype with a short stalk, which brings flowers closer to the ground, would increase the temperature of its developing flowers by absorbing more radiant heat coming off the ground. Similarly, spike length plasticity might allow a genotype to adjust the heat loss from a spike. For example, a longer spike would have a lower surface area to volume ratio, which would increase the temperature of its developing flowers. For these reasons, I examined plasticity in all these traits: flowering onset, flowering duration, rate of reproduction, stalk length, and spike length.

CHAPTER II

METHODS

Genotype Selection

I initially selected 53 *P. lanceolata* genotypes within their native European range based on latitude, floral reflectance plasticity at 850nm, and altitude. These genotypes were the progeny of half-sib crosses of maternal parents collected from wild populations in 2000 (Lacey et al 2010). All my experimental genotypes were derived from populations at altitudes <250m. In order to minimize the confounding effects of latitude and reflectance plasticity, I selected genotypes that varied in floral reflectance plasticity across all latitudes (Fig. 1A). Latitude for genotypes ranged from 41°N to 62°N (Italy to Scandinavia). Floral reflectance plasticity for genotypes ranged from -10% to 60% at 850nm.

Transplant Sites

I selected two environments within the introduced range of *P. lanceolata* for my field experiment. These sites differed in temperature and length of the reproductive season (Fig. 1B). The North Carolina Agricultural and Technical State University Farm (36° 06'N, 79 73'W; elevation 272m) hereafter called NCA&T, is located on the eastern boundary of Greensboro, NC on land that is used for growing crops such as corn and tomatoes. Mountain Lake Biological Station (37° 37'N, 80 52'W; elevation 1181m),

hereafter called MLBS, is a research facility located on Salt Pond Mountain in Pembroke, VA. The reproductive season for *P. lanceolata* at NCA&T is, on average, long and warm, beginning in early April and extending into early September. At MLBS, the reproductive season is, on average, short and cool, beginning in late May and early June and extending into early September. For each site, I prepared field plots that measured approximately 3x3 meters. At NCA&T, the field plot was located in a regularly mowed area where *P. lanceolata* along with forbs and grasses grow naturally. At MLBS, the field plot was located inside a deer enclosure where *P. lanceolata* grows naturally.

Experimental Design

For each genotype, I made clones by dividing a rosette. A total of 4 growth chambers were used and clones were randomly assigned positions so that no more than 2 clones per genotype were in the same chamber. Clones were grown in growth chamber settings of 20°C, 10h day/15°C, 14h night to promote vegetative growth for approximately 2 months. Clones were watered daily and received ½ -strength Hoagland's solution 3X/wk. Clones of similar size were used in the experiment.

In March 4th and April 15th of 2011, I transplanted three clones per genotype from growth chambers into field plots at each site of NCA&T and MLBS, respectively. Clones were randomly assigned a position in the plot where plants were 20 cm apart. At both sites, I recorded and marked biweekly spike production for each clone. Upon maturity, spikes were collected and recorded for stalk length and spike length.

I estimated seed production per clone by first estimating seed production per spike. To do this, I sampled spikes from different clones at each transplant site. I

weighted each spike and counted the number of seeds per spike (MLBS: N=53; NCA&T: N=69). Then, I fit a linear regression (PROC REG) model to estimate seed production for all collected spikes based on spike weight. There was a statistically positive association between spike weight and seed production (MLBS seeds model= 0+380.6891(spike weight), r^2=0.82198; NCA&T seeds model= 0+403.4024(spike weight), r^2=0.77335; Fig. 2). Grasshopper damage was negligible at both sites. Estimated seed numbers for all spikes produced per clone were then summed for each two-week period. For each genotype, cumulative seed production per two-week period was established by summing the previous seed production per clone and averaging over all clones (1-3). Final seasonal seed production for each genotype was established by averaging total seasonal seed production per clone over all clones.

In 2012, I followed the same procedure but recorded data only at MLBS because a tilling accident destroyed the plot at NCA&T. I used the same model from 2011 to estimated cumulative and final seed production for each genotype at MLBS in 2012.

Measurements of plasticity

I used genotypes whose floral reflectance plasticity had been established (Lacey et al 2010). Plasticity for each genotype was calculated by taking the difference in percent floral reflectance between spikes that developed at warm (27°C day / 20°C night) temperatures and the percent floral reflectance at cool (15°C day / 10°C night) temperatures (see Lacey and Herr 2005 for complete methodology).

Flowering onset for each clone was measured as the Julian week when the first spike was produced. To estimate plasticity in flowering onset for each genotype, I took

the difference between mean onset time of clones at NCA&T minus mean onset time of clones at MLBS (Fig. 3A).

Flowering duration for each clone was measured as the number of Julian weeks between the first and last spike produced. To estimate plasticity in flowering duration for each genotype, I took the difference between mean duration time of clones at NCA&T minus mean duration time of clones at MLBS (Fig. 3B).

Reproductive rate for each clone was measured by using the median week of spike production. Median week measures the number of weeks that it takes for a clone to produce half of its spikes since the first spike was produced. This serves as a measure of the rate of spike development within a reproductive season. To estimate plasticity in median week for each genotype, I took the difference between median week of spike production of clones at NCA&T minus median week of spike production of clones at MLBS (Fig. 3C).

Stalk length per spike was measured when each spike was collected. Because multiple spikes are produced per plant, I examined if stalk length varied throughout the reproductive season. Using linear mixed models (PROC MIXED), I assessed the mean stalk length of the first 20 spikes produced at each site. I found that stalk length varied (Fig. C1). Therefore, I used the average of the first 20 stalks to estimate plasticity per clone. To estimate plasticity in stalk length for each genotype, I took the difference in mean stalk length of clones at NCA&T minus mean stalk length of clones at MLBS (Fig. 3D).

Spike length per spike was measured when each spike was collected. Spike length estimates the number of flowers in a spike. Flower number increases with spike length (Fig. C2). More flowers can potentially increase seed production. Because multiple spikes are produced per plant, I examined if spike length varied throughout the reproductive season. Using linear mixed models (PROC MIXED), I assessed the mean spike length of the first 20 spikes produced at each site. I found that spike length varied (Fig. C3). Therefore, I used the average of the first 20 spikes to estimate plasticity per clone. To estimate plasticity in spike length for each genotype, I took the difference in mean spike length of clones at NCA&T minus mean spike length of clones at MLBS (Fig. 3E).

Magnitude and directionality of plasticity

By subtracting NCA&T minus MLBS for all my reproductive traits, I measured the magnitude and directionality of plasticity. For each reproductive trait, the magnitude of plasticity represented how much a particular genotype could alter its response. For example, a genotype with a value of 3.2cm for spike length plasticity could alter its spike length by 3.2cm. Because I did not take the absolute difference between sites, I was also able to measure the direction of plasticity. The directionality of plasticity (- or + value) indicated at which site a particular genotype had the higher value. Positive values meant that a trait had a higher value at NCA&T, whereas negative values meant that a trait had higher value at MLBS.

Statistical Analyses

In 2011, eight genotypes did not survive the transplanting and only 34 of the remaining 45 genotypes produced spikes at MLBS. At NCA&T, all genotypes survived the transplanting and 48 of the 53 genotypes produced spikes. A total of 34 genotypes flowered in MLBS and NCA&T in 2011. Therefore, I calculated plasticity values for these genotypes and used them in my analysis in 2011.

In 2012, I performed my statistical analyses on data only from MLBS. A total of 24 genotypes flowered at MLBS in 2012. Of these genotypes, five were mole damaged and six had not flowered in 2011. Therefore, I did not have plasticity data for six genotypes. Consequently, the sample size for 2012 was 13 genotypes.

All statistical analyses were performed in SAS version 9.2 (PROC MIXED). To determine whether flowering onset, flowering duration, median week of spike production, stalk length and spike length were phenotypically plastic, I examined each trait individually for a main effect of transplant site and for genotypic variation in plasticity for each trait. In order to assess if plasticity in any trait and source latitude were associated, I conducted a Pearson correlation test (PROC CORR) on all independent traits and source latitude.

To determine the effects of plasticity on reproductive success, I performed two types of analyses. First, I used linear mixed models to examine the effects of Julian week and each plastic trait individually on the temporal patterns of cumulative spike and seed production. Spike and seed production were log-transformed to improve normality.

Second, I performed phenotypic-selection analyses to determine if plasticity on each trait showed an effect on total seasonal spike and seed production. Significant effects would provide evidence of selection on that trait. The selection analyses included source latitude as a covariate. Linear and quadratic regression models were used. Relative fitness was calculated by dividing final seasonal spike, or seed, production by the final mean seasonal production for each site. Each plasticity value was standardized so that the mean = 0 and variance = 1 (Lande and Arnold 1983). Standard linear (β) and quadratic (γ) selection gradients were estimated, and the parameter estimate for γ was doubled (cf. Stinchcombe et al 2008). A statistically significant linear selection gradient (β) provides evidence for directional selection, whereas a significant quadratic gradient (γ) provides evidence of curvature in the fitness function and evidence of stabilizing or disruptive selection.

CHAPTER III

RESULTS

2011

The reproductive seasons differed between NCA&T and MLBS. The reproductive season spanned Julian weeks 17-31 (April-August) at NCA&T and 25-34 (June-September) at MLBS. At NCA&T, three genotypes were flowering by week 17, and all were flowering by week 25. At MLBS, twenty genotypes were flowering by week 25, and all were flowering by week 31. On average, genotypes at NCA&T began flowering earlier, flowered for longer, had a longer developmental rate, and produced longer spikes than the genotypes at MLBS (Fig. 4). At NCA&T, relative to MLBS, flowering began 4.75 weeks earlier (17.99% change), flowering lasted 1.6 weeks longer (46.70% change), rate of spike development slowed by 1.7 weeks (154.14% change), and spike length increased by 0.20 cm (11.7% change). In contrast, average stalk length did not statistically differ between sites.

In addition to reflectance plasticity, genetic variation in plasticity was observed in flowering onset, stalk length, and spike length (Table 1). These traits showed highly significant genotype by transplant site interactions. Therefore, I was able to examine the effects of plasticity in reflectance, onset, stalk length, and spike length on reproductive success in 2011. Of these traits only flowering onset plasticity showed statistically

significant phenotypic correlations with source latitude (Table 2). As the source latitude of genotypes increased, plasticity in flowering onset increased. Also, genotypes with greater plasticity for stalk length were more plastic for spike length.

Plasticity affected the temporal pattern of spike and seed production. Linear models, which did not include source latitude as a covariate, showed that the effects varied by trait, site, fitness proxy, and time during the reproductive season (Table 3). Using cumulative seed production as the fitness proxy, genotypes showed several patterns. At MLBS, as the reproductive season progressed more plastic genotypes for stalk and spike length produced more seeds than less plastic genotypes (Fig. 5A, 6A). At NCA&T, more plastic genotypes for flowering onset produced more spikes and seeds than less plastic genotypes (Fig. 7B). The strength of these linear effects increased as the reproductive season progressed at each site.

Phenotypic-selection analyses, which included source latitude as a covariate, examined both linear and nonlinear effects on total seasonal spike and seed production and showed evidence of statistically significant effects of plasticity only at MLBS. The effects and patterns of selection, however, varied by trait and fitness proxy (Table 4). Using total seasonal seed production as the fitness proxy, two types of selection were observed. First, the negative quadratic selection gradient showed evidence of stabilizing selection favoring an intermediate value of plasticity for flowering onset (Fig. 7A). Genotypes with intermediate plasticity had the greatest reproductive success. Second, negative linear selection gradients showed evidence of directional selection favoring stalk and spike length plasticity at MLBS (Fig. 5A, 6A). The negative values indicated that

genotypes producing longer stalks and spikes at MLBS than at NCA&T had greater reproductive success at MLBS than did genotypes having the same magnitude of plasticity but in the opposite direction. No statistically significant effect of source latitude was observed on reproductive success (Table 4).

2012

At MLBS, the reproductive season spanned Julian weeks 20-36 (May-September). No data were collected at NCA&T because the plot was destroyed. Three genotypes were flowering by week 20, and all except for one genotype, which flowered in week 34, were flowering by week 28.

Similar to 2011, plasticity affected the temporal pattern of seed production at MLBS in 2012. Linear models, which did not include source latitude as a covariate, showed that the effect varied by trait, fitness proxy, and time during the reproductive season (Table 5). Genotypes having greater plasticity in stalk and spike length produced more seeds and this advantage increased progressively over the reproductive season (Fig. 5C, 6C). There was no effect on spike production.

Phenotypic-selection analyses showed evidence of statistically significant selection for only stalk length plasticity and source latitude (Table 4). Stalk plasticity affected seed production at MLBS. The negative linear selection gradient indicated that genotypes producing longer stalks at MLBS than at NCA&T had greater reproductive success than did genotypes having the same magnitude of plasticity but in the opposite direction (Fig. 5C). In addition, there was statistical evidence that source latitude significantly affected reproductive success. As source latitude of the genotypes increased,

final seasonal seed production increased (Fig. 8). A similar but not statistically significant trend is also observed for the effects of source latitude on total seasonal spike production.

CHAPTER IV

DISCUSSION

Invasive species are widely characterized as having more phenotypic plasticity than non-invasive species (e.g. Richards et al 2006; Davidson et al 2011). Many authors have argued that this plasticity enables invasive species to colonize novel environments (Mal and Lovett-Doust 2005; Geng et al 2007; Funk 2008). My experiment is the first of which I am aware that directly test this hypothesis by comparing the reproductive success of genotypes that differ in multiple plasticities in natural habitats. My experiment also provides new information about the plasticity of reproductive traits and phenotypic correlations among plasticities and source latitude.

My data show that multiple reproductive traits in *P. lanceolata* express phenotypic plasticity. Flowering onset, flowering duration, rate of spike development, stalk length, and spike length showed strong evidence of plasticity in the field (Table 1). However, stalk length, in context to the other traits, showed no difference in mean stalk length between sites but did show that genotypes varied in their site responses. These results are concordant with a growth chamber experiment (Blank and Lacey unpubl.) and suggest that in *P. lanceolata*, many reproductive traits are temperature-sensitive and that genetic variation in thermal plasticity exists for flowering onset, stalk length, and spike length.

My data provide support for the hypothesis that plasticity in several reproductive traits, specifically flowering onset, stalk length, and spike length, can improve reproductive success in novel environments. Linear models, which examined cumulative spike and seed productive over time, showed that the effects of plasticity increased progressively over the reproductive season. Also, phenotypic-selection analyses on total seasonal seed production showed evidence of stabilizing selection for flowering onset and directional selection favoring plasticity in stalk length and spike length.

However, the advantages of plasticity on reproductive success appear to be site-specific. At NCA&T, which has, on average, a warm and long reproductive season, phenotypic-selection analyses showed no evidence of selection for plasticity in any trait. In contrast, at MLBS, which has, on average, a cool and short reproductive season, phenotypic-selection analyses showed evidence of directional selection favoring genotypes that produce longer stalks and spikes at presumably cooler environments, rather than the reverse. In addition, there was evidence of stabilizing selection for the ability to alter flowering onset. Genotypes with intermediate onset plasticity values produced the most seeds.

One possible explanation for these site-specific responses is that plasticity allows for partial thermoregulation. Thermoregulation allows a genotype to respond to external temperatures and maintain internal body temperatures closer to optimum. Lacey et al (2010) proposed that thermoregulation should be more advantageous in thermally variable cooler and shorter reproductive seasons than in warmer and longer reproductive seasons. Consistent with this hypothesis, onset plasticity, which might allow a genotype

to alter the timing of its reproductive season so that flowering begins under favorable environmental temperatures, was not selected in a warm and long reproductive season (i.e. NCA&T). Perhaps the environmental temperatures were already close to the optimum. Conversely, in a cool and short reproductive season (i.e. MLBS), there was selection for onset plasticity. This flexibility could enable genotypes to delay flowering if ambient temperatures are too cold even after the critical photoperiod cue for flowering has arrived. Extreme onset plasticity, however, could be detrimental because the reproductive window is small and waiting for the optimum environmental conditions could result in not flowering at all. This would explain why there was selection on the intermediate values of onset plasticity at MLBS.

At MLBS, there was evidence for directional selection favoring longer spikes at MLBS than at NCA&T. This suggests that there was selection for plants to lengthen spikes at cooler temperatures. Spike length plasticity could allow for partial thermoregulation. Spike shape approximates a prolate ellipsoid. Therefore, as a spike lengthens the surface to volume ratio decreases, which reduces heat loss. At a warm and long reproductive season, spike length plasticity is not selected perhaps because environmental temperatures are adequate. Also, the ability to produce a longer spike in a cool environment could be advantageous because more flowers and potentially more seeds can be produced with fewer resources allocated to stalk tissue. Why larger stalk lengths are also selected at the cooler and shorter reproductive season is unknown at this time. However, there was a strong positive phenotypic correlation between stalk length plasticity and spike length plasticity. Perhaps selection for spike length plasticity also

results in stalk length plasticity. Future studies could examine whether these correlations are genetically based.

I also found evidence that the effects of plasticity can vary between reproductive years. At MLBS, the observed advantages of onset and spike length plasticity in 2011 disappeared in 2012. However, source latitude positively affected reproductive success in 2012 at MLBS. It is possible that plants derived from high latitude populations (Fig. C4) accumulated more resources during the winter, which resulted in more seeds. I do not know whether a similar pattern would have appeared at NCA&T in 2012 because the plot was destroyed. One might ask why source latitude had no effect in 2011 at both sites. If source latitude is associated with vegetative traits that affect resource accumulation, then these traits would require more time (i.e. from fall-spring) to show their possible effects.

Although I focused on the plasticity of reproductive traits, experiments (e.g. van Tienderen and van Hinsberg 1996) have shown that *P. lanceolata* can express plasticity in vegetative traits. Perhaps the effect of source latitude in the second year of the experiment reflected selection for vegetative traits that are associated with latitude. Future studies might examine which vegetative traits, if any, are associated with source latitude. From this perspective my study raises several questions that would be worth pursuing. Do other unmeasured, e.g. vegetative traits, show genetic variation in plasticity? If so, does vegetative plasticity affect colonization success? What are the combined effects of reproductive plasticity and vegetative plasticity on colonization success? Colonization of a new environment requires that a plant successfully survives and reproduces in that environment. If plasticity in vegetative traits can improve survival,

then there should be a selective advantage for vegetative plasticity. Lastly, does plasticity influence reproductive success independently of the mean value of a trait in a given environment?

Presently, my results generally support the argument that plasticity facilitates the colonization of novel environments. The benefits of plasticity on reproductive success, however, depend on the new environment and the plasticity of the reproductive trait in question. My study highlights the need to better understand how selection acts on plasticity in multiple vegetative and reproductive traits.

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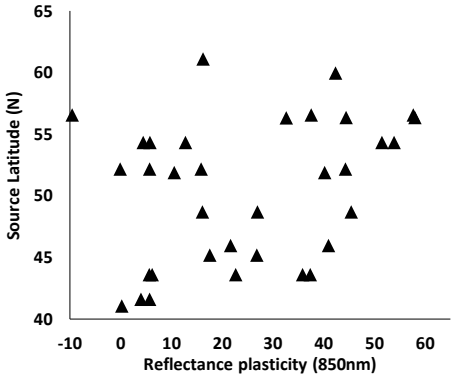
APPENDIX A

FIGURES

Figure 1. Experimental genotypes and transplant sites.

Selected genotypes used in the experiment varied in (A) source latitude (41˚N-62˚N) and floral reflectance plasticity (-10nm-60nm) across Europe. Genotypes were transplanted into (B) two transplant sites. 30-year mean monthly temperature for NCA&T (black squares, solid line) and 10-year mean monthly temperature for MLBS (open circle, dashed line). The arrows at the bottom depict the length of the flowering season for NCA&T (black line) and MLBS (dashed line).

A)



B)

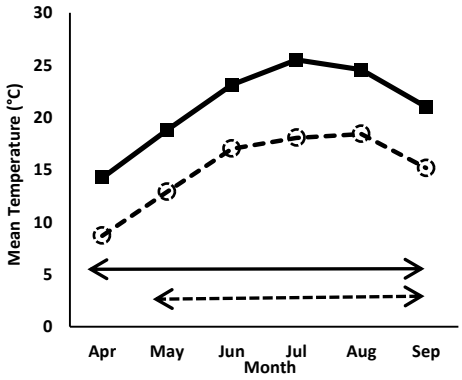
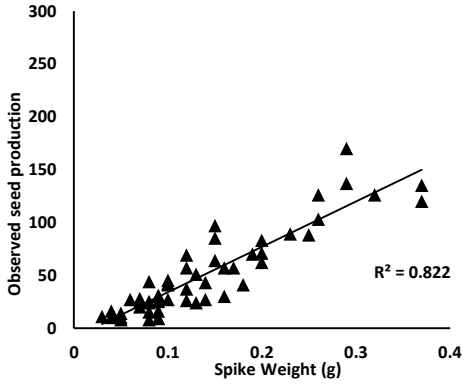


Figure 2. Estimated seed production models for 2011 and 2012.

Linear relationship for transplant site (A) MLBS and (B) NCA&T between spike weight (g) and seed production (MLBS: N=53; NCA&T: N=69). The linear regression was used to developed models to estimate seed production. The models were 0+380.6891(spikeweight) for MLBS and 0+403.40247(spikeweight) for NCA&T.

A)



B)

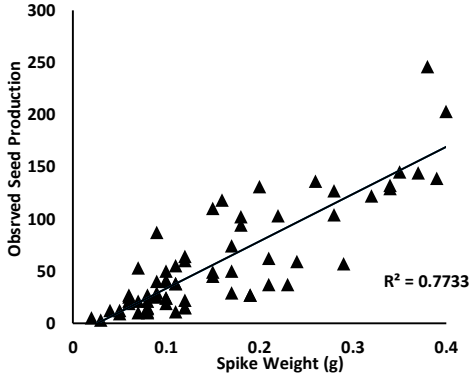


Figure 3. Traits of genotypes at NCA&T and MLBS in 2011.

A genotype's (A) flowering onset, (B) flowering duration, (C) median week of spike production, (D) stalk length, and (E) spike length at each transplant site. For each trait, values were calculated by taking the mean of clones (1-3) at each site. The diagonal line in each graph indicates the theoretical line of non-plasticity, that is, no difference in trait value between a genotype planted at NCA&T and MLBS.

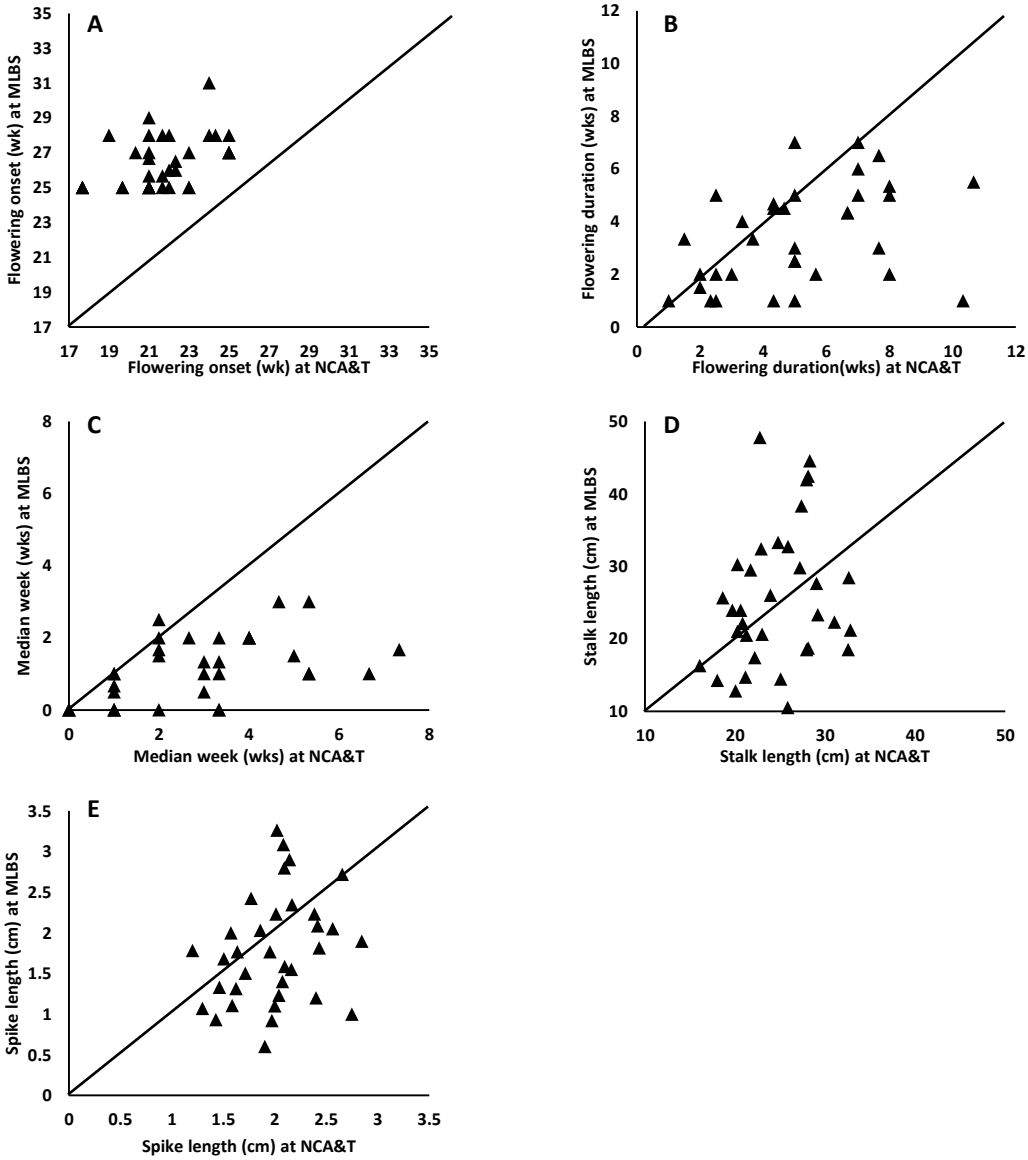


Figure 4. Trait means at NCA&T and MLBS in 2011.

Trait mean for (A) flowering onset, (B) flowering duration, (C) median week of spike production, (D) stalk length, and (E) spike length (±1SE) at each transplant site.

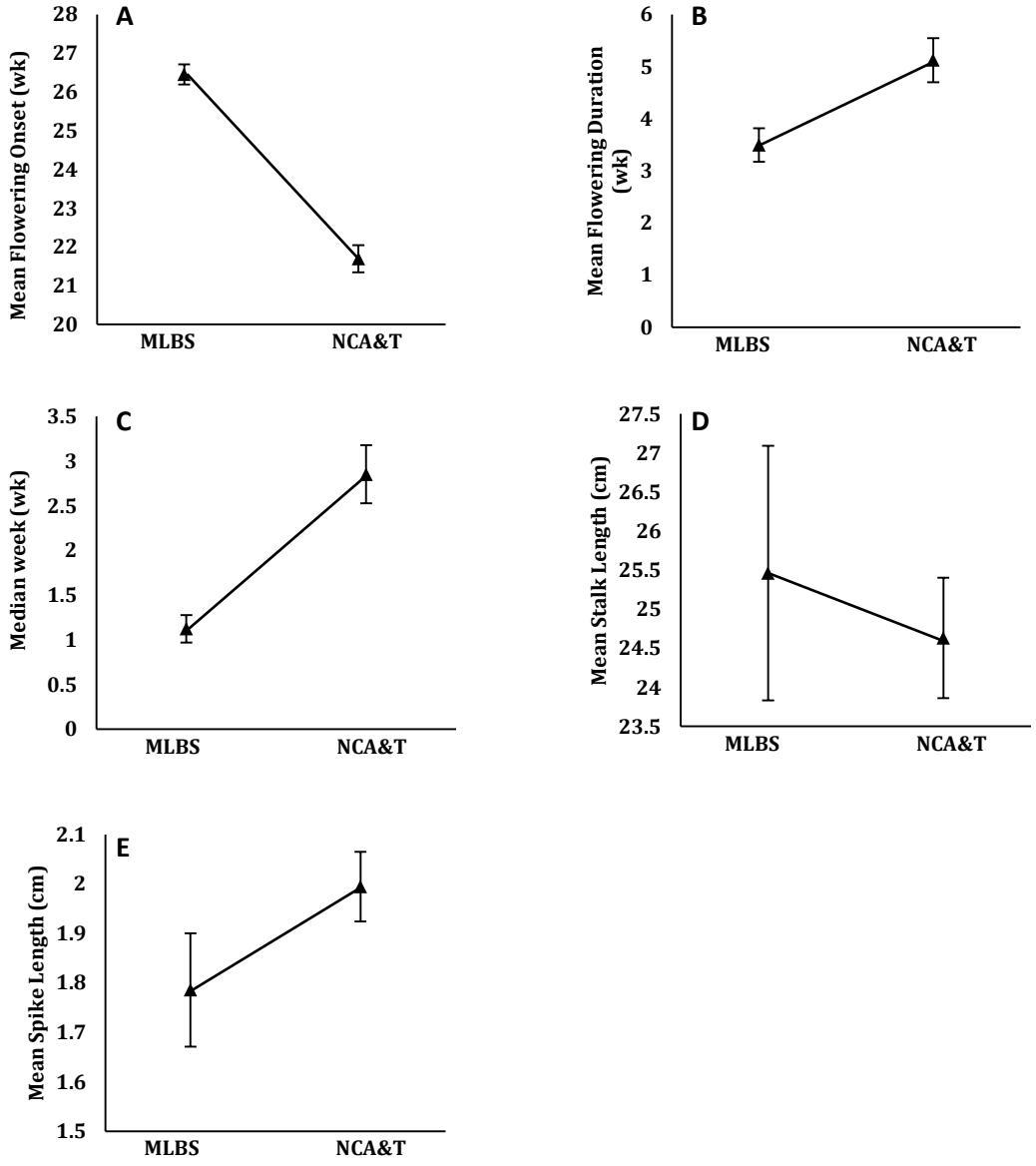


Figure 5. Effects of 2011 stalk length plasticity on cumulative spike and seed production.

Second-order polynomial relationships for (A) MLBS 2011, (B) NCA&T 2011, and (C) MLBS 2012 between stalk length plasticity and cumulative spike and seed production per genotype (1-3 clones/genotype/wk). Weeks 19, 25, 26 (rhombus, thin black line), 25, 29, 30 (square, dashed line), and 31, 34, 36 (triangle, thick black line).

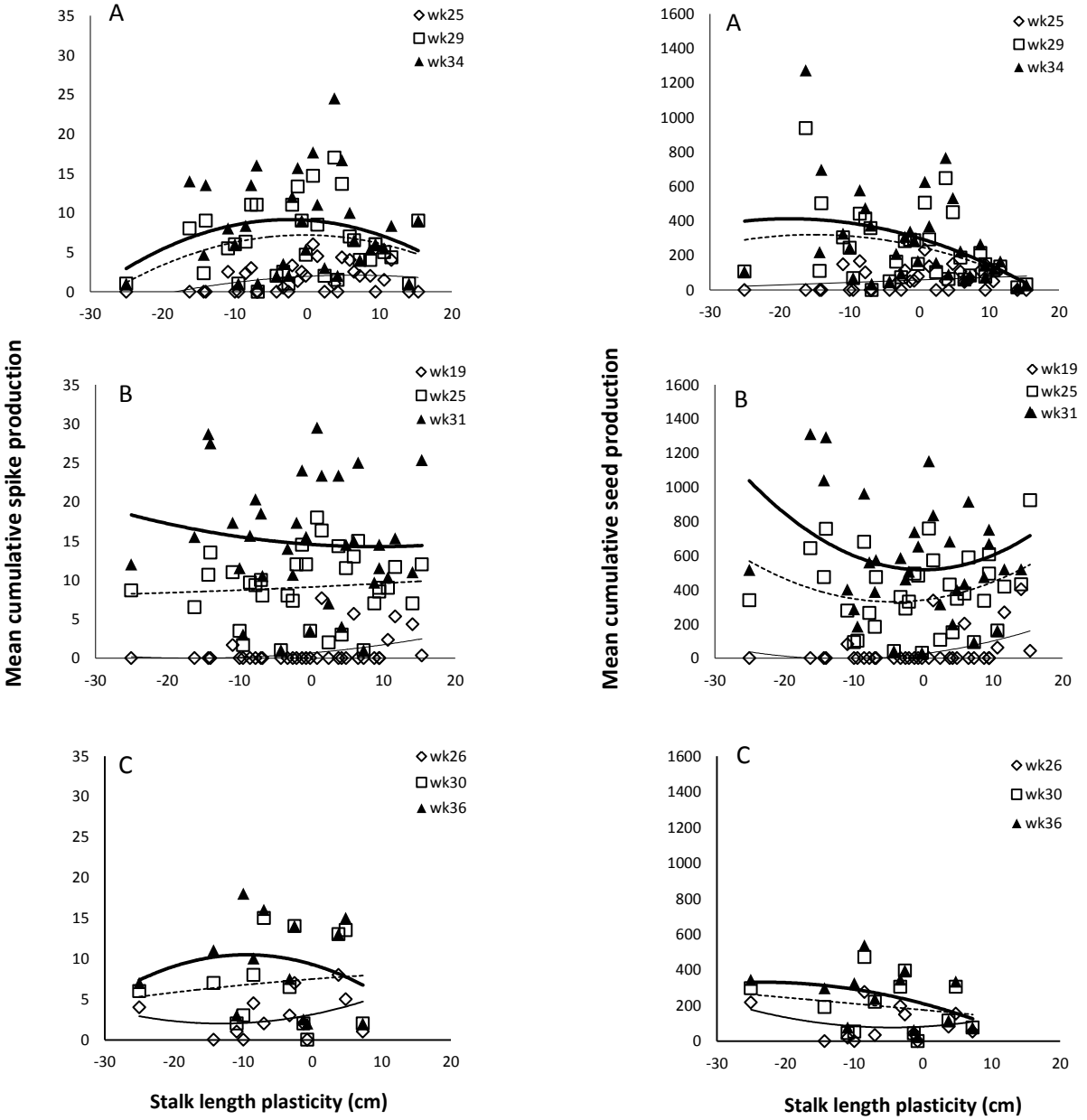


Figure 6. Effects of 2011 spike length plasticity on cumulative spike and seed production.

Second-order polynomial relationships for (A) MLBS 2011, (B) NCA&T 2011, and (C) MLBS 2012 between spike length plasticity and cumulative spike and seed production per genotype (1-3 clones/genotype/wk). Weeks 19, 25, 26 (rhombus, thin black line), 25, 29, 30 (square, dashed line), and 31, 34, 36 (triangle, thick black line).

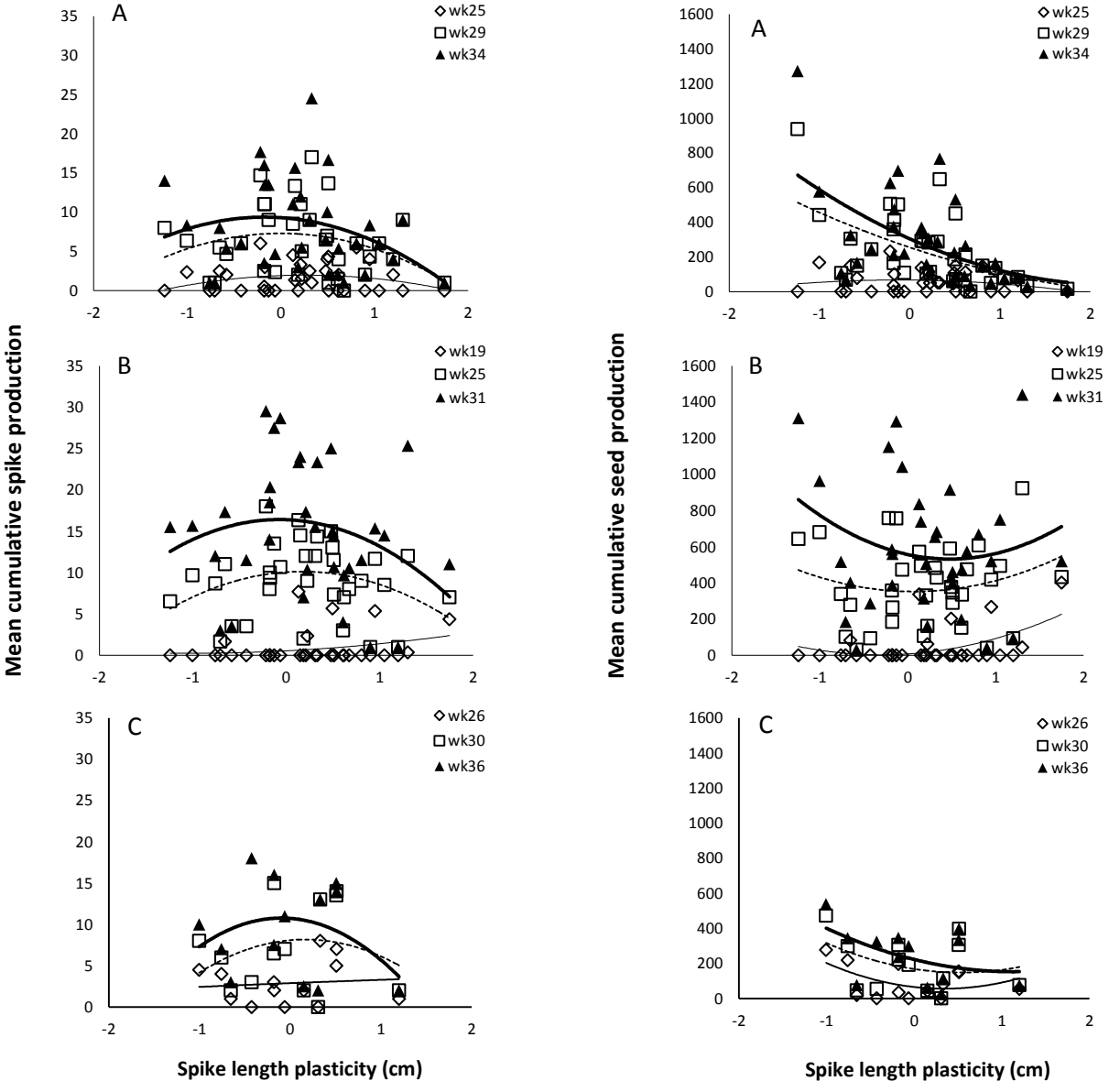


Figure 7. Effects of 2011 onset plasticity on cumulative spike and seed production.

Second-order polynomial relationships for (A) MLBS 2011, (B) NCA&T 2011, and (C) MLBS 2012 between onset plasticity and cumulative spike and seed production per genotype (1-3 clones/genotype/wk). Weeks 19, 25, 26 (rhombus, thin black line), 25, 29, 30 (square, dashed line), and 31, 34, 36 (triangle, thick black line).

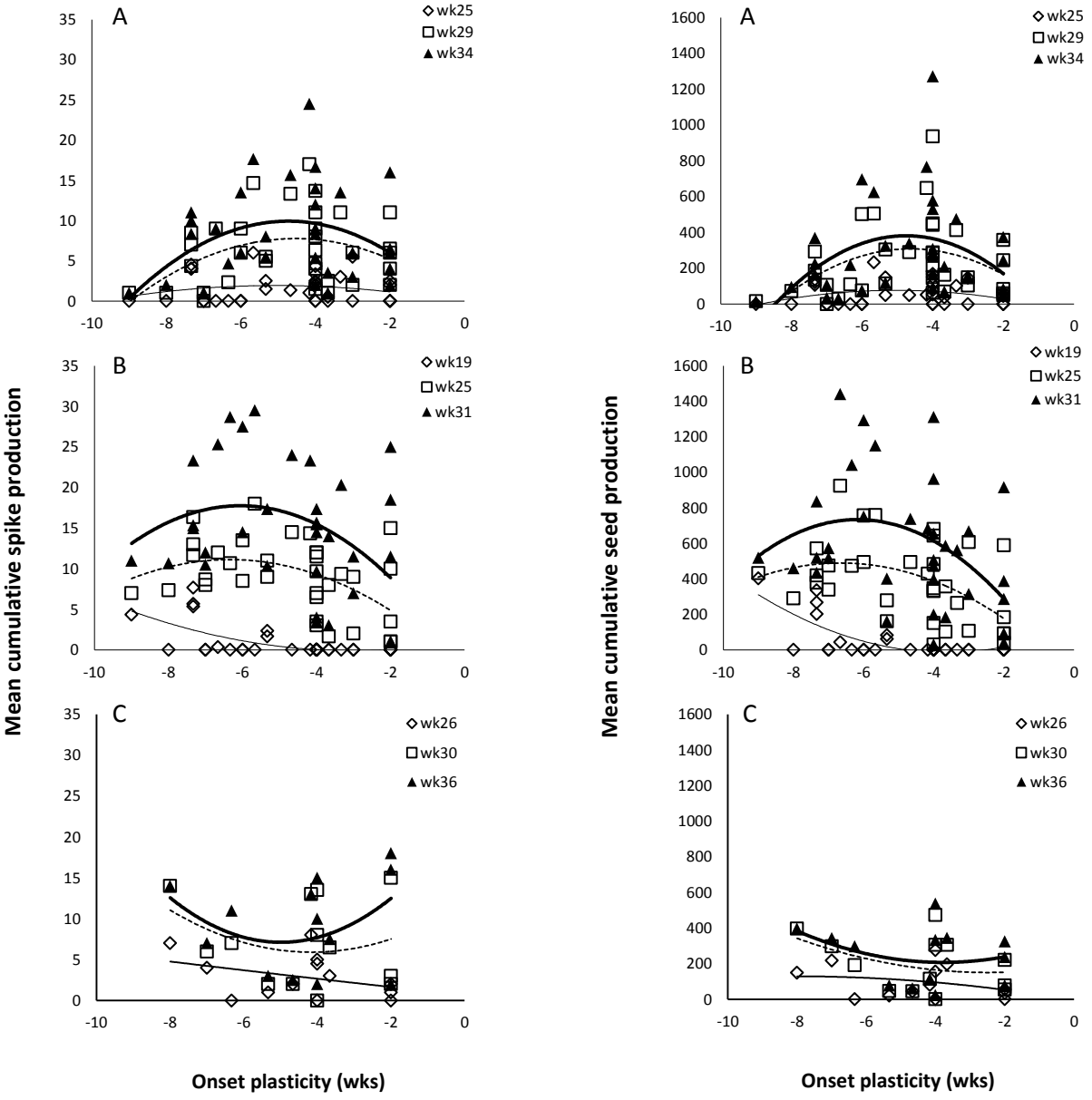
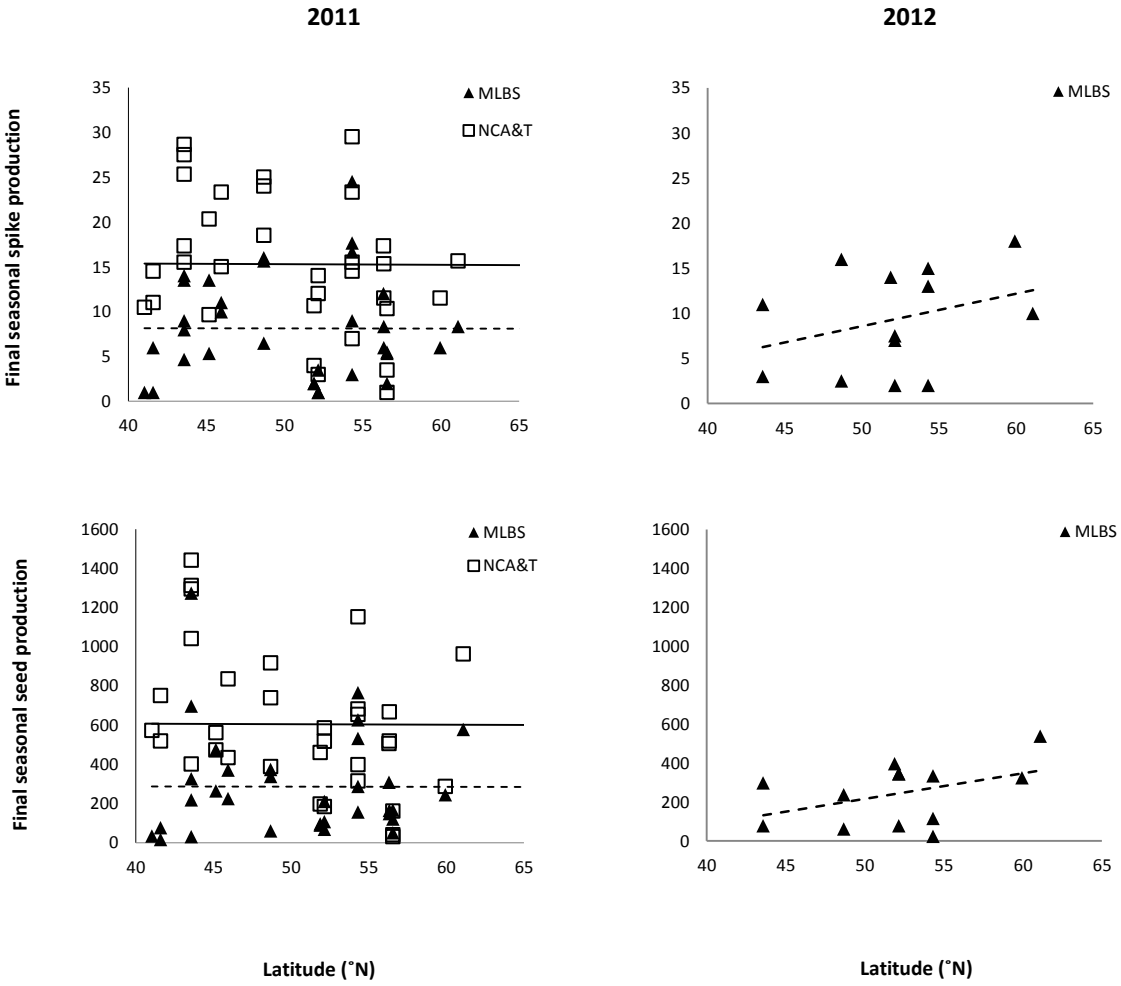


Figure 8. Effects of source latitude on reproductive success in 2011 and 2012.

Linear relationship for total seasonal spike and seed production for NCA&T and MLBS in 2011 and 2012. MLBS is shown with solid triangle, dashed line. NCA&T is shown with open squares, solid line.



APPENDIX B

TABLES

Table 1. Plasticity of reproductive traits.

Effects of genotype, transplant site, and their interaction on mean flowering onset, flowering duration, median week of spike production, stalk length, and spike length. P values are shown, n=34.

Effect	Flowering Onset	Flowering Duration	Median week	Stalk length	Spike length
Genotype	<0.0001	0.0253	0.0091	<0.0001	<0.0001
Site	<0.0001	0.0003	<0.0001	0.2854	0.0045
Genotype X site	<0.0001	0.4344	0.1487	<0.0001	0.0002

Table 2. Correlation between independent variables.

Pearson correlation coefficients between a genotype's source latitude, reflectance plasticity, onset plasticity, stalk length plasticity, and spike length plasticity.

	Source latitude		Reflectance plasticity		Onset plasticity		Stalk length plasticity		Spike length plasticity	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
Source Latitude										
Reflectance plasticity	0.30479	*0.0797*								
Onset plasticity	0.45494	*0.0069*	0.11421	*0.5201*						
Stalk length plasticity	0.02969	*0.8676*	0.32704	*0.059*	-0.10027	*0.5726*				
Spike length plasticity	-0.20045	*0.2557*	0.01257	*0.9437*	-0.23515	*0.1807*	0.78917	*<0.0001*		

Table 3. Effects of plastic traits on reproductive success in 2011.

Linear models on the individual effects of reflectance plasticity, onset plasticity, stalk length plasticity, and spike length plasticity by Julian week on cumulative spike and seed production at each transplant site in 2011.

MLBS	**Reflectance Plasticity**				**Onset Plasticity**				**Stalk length Plasticity**				**Spike length Plasticity**			
	Spikes		Seeds		Spikes		Seeds		Spikes		Seeds		Spikes		Seeds	
Julian week	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
25	0.02653	0.5045	0.3176	0.8509	-0.02304	0.9591	-0.1775	0.9925	0.06982	0.4198	1.4084	0.6904	0.1304	0.915	-18.6656	0.6992
27	0.04475	0.2605	0.5192	0.7586	0.08364	0.8522	5.2561	0.7813	0.03665	0.6718	-2.7123	0.4432	-0.1984	0.871	-80.1407	0.0975
28	0.0409	0.3037	0.1516	0.9285	0.3323	0.4595	11.6351	0.5389	0.03082	0.7217	-5.4082	0.1266	-0.371	0.7614	-120.06	0.0132
29	0.04242	0.2861	-0.114	0.9462	0.4225	0.347	14.874	0.4322	0.02347	0.7862	-6.9489	0.0499	-0.6381	0.6016	-142.56	0.0033
30	0.05296	0.1831	-0.3339	0.8433	0.2809	0.5317	12.5687	0.5068	0.04124	0.6337	-7.6552	0.0308	-0.5122	0.6751	-159.62	0.001
31	0.04277	0.2822	-0.6879	0.6839	0.2408	0.5919	11.2035	0.554	0.02505	0.7722	-8.4928	0.0166	-0.6918	0.5713	-166.82	0.0006
32	0.04447	0.2635	-0.6266	0.7107	0.2788	0.5348	11.7996	0.5332	0.01339	0.877	-9.0794	0.0105	-0.8689	0.4771	-175.93	0.0003
34	0.0393	0.323	-0.7157	0.6719	0.2268	0.6136	10.6833	0.5726	-0.01494	0.8629	-10.177	0.0042	-1.1415	0.3504	-188.72	0.0001
NCA&T	**Reflectance Plasticity**				**Onset Plasticity**				**Stalk length Plasticity**				**Spike length Plasticity**			
	Spikes		Seeds		Spikes		Seeds		Spikes		Seeds		Spikes		Seeds	
Julian week	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
17	0.01096	0.7691	0.5321	0.7715	-0.2466	0.4947	-9.9474	0.5708	0.02238	0.7513	1.0039	0.7713	0.1802	0.859	8.9233	0.8572
19	0.02128	0.569	0.3763	0.8373	-0.642	0.0757	-36.9069	0.0357	0.07695	0.2759	4.5761	0.1855	0.8829	0.3843	67.2883	0.175
21	0.02909	0.4362	0.6314	0.7304	-0.7779	0.0315	-43.5734	0.0132	0.08307	0.2396	5.5543	0.1081	0.6494	0.5222	66.0199	0.1833
23	0.0292	0.4344	0.6376	0.7279	-0.6916	0.0557	-39.7647	0.0237	0.0658	0.3515	4.0956	0.2359	0.4785	0.6372	47.3376	0.3399
25	0.03685	0.324	0.3252	0.8591	-0.6449	0.0744	-37.5097	0.0328	0.05423	0.4425	3.0694	0.3743	0.4838	0.6335	47.7513	0.3357
27	0.02027	0.5873	-0.6289	0.7315	-0.7382	0.0412	-40.8596	0.0201	-0.0213	0.7629	-1.8535	0.5916	-0.03833	0.9699	-6.6633	0.8931
29	0.007054	0.8502	-0.9064	0.6209	-0.6869	0.0574	-40.3082	0.0219	-0.04056	0.5657	-1.9989	0.5628	-0.2171	0.8306	-8.4663	0.8644
30	0.004666	0.9006	-0.9842	0.5912	-0.6855	0.0579	-41.5972	0.018	-0.04869	0.4905	-1.9452	0.5734	-0.2593	0.7983	-5.97	0.9042
31	0.004666	0.9006	-0.9842	0.5912	-0.6855	0.0579	-41.5972	0.018	-0.04869	0.4905	-1.9452	0.5734	-0.2593	0.7983	-5.97	0.9042

Table 4. Selection coefficients for plasticity in reproductive traits in 2011 and 2012.

Selection coefficients for reflectance plasticity, onset plasticity, stalk plasticity, and spike plasticity in *Plantago lanceolata*. Fitness proxies in (A) 2011 were calculated by using total seasonal spike and seed production at week 34 and 31 for MLBS and NCA&T, respectively. Fitness proxies in (B) 2012 were calculated by using total seasonal spike and seed production at week 36 for MLBS. Source latitude was included in each model as a covariate. Note: n=34 in 2011 and n=13 in 2012.

A) 2011								
MLBS	**Spikes**				**Seeds**			
Trait	Linear (B)	*P*	Quadratic (2y)	*P*	Linear (B)	*P*	Quadratic (2y)	*P*
Reflectance plasticity	0.07758	*0.483*	-0.3026	*0.242*	-0.00864	*0.9511*	-0.5022	*0.1232*
Source latitude	0.004817	*0.8547*	-	-	0.01533	*0.6185*	-	-
Onset plasticity	-0.06205	*0.6093*	0.3074	*0.0998*	-0.02802	*0.8527*	-0.471	*0.0464*
Source latitude	0.00471	*0.8593*	-	-	-0.00148	*0.9589*	-	-
Stalk length plasticity	-0.05722	*0.6385*	-0.18078	*0.3393*	-0.301	*0.0442*	-0.15262	*0.5103*
Source latitude	0.00396	*0.8759*	-	-	0.008196	*0.7728*	-	-
Spike length plasticity	-0.1096	*0.3556*	0.16954	*0.4398*	-0.3971	*0.0056*	0.10826	*0.5867*
Source latitude	0.001872	*0.9429*	-	-	-0.0102	*0.7229*	-	-
NCA&T	**Spikes**				**Seeds**			
Trait	Linear (B)	*P*	Quadratic (2y)	*P*	Linear (B)	*P*	Quadratic (2y)	*P*
Reflectance plasticity	0.05769	*0.4755*	-0.1889	*0.3216*	0.07261	*0.4417*	-0.3264	*0.146*
Source latitude	-0.02047	*0.2517*	-	-	-0.0272	*0.1947*	-	-
Onset plasticity	-0.1523	*0.0852*	-0.17602	*0.2306*	-0.1066	*0.3247*	-0.2034	*0.2718*
Source latitude	-0.00611	*0.7571*	-	-	-0.0214	*0.3347*	-	-
Stalk length plasticity	-0.03143	*0.7187*	0.02752	*0.8435*	0.01674	*0.8714*	0.1642	*0.3081*
Source latitude	-0.01847	*0.312*	-	-	-0.02396	*0.2577*	-	-
Spike length plasticity	-0.04522	*0.5862*	-0.2206	*0.0962*	-0.0527	*0.6118*	0.09986	*0.5355*
Source latitude	-0.02393	*0.1953*	-	-	-0.02981	*0.1722*	-	-
B) 2012								
MLBS	**Spikes**				**Seeds**			
Trait	Linear (B)	*P*	Quadratic (2y)	*P*	Linear (B)	*P*	Quadratic (2y)	*P*
Reflectance plasticity	-0.06437	*0.7558*	0.1454	*0.8535*	-0.1943	*0.3295*	0.2776	*0.7038*
Source latitude	0.04627	*0.3048*	-	-	0.08161	*0.056*	-	-
Onset plasticity	0.09011	*0.6606*	0.3378	*0.2385*	-0.2026	*0.3528*	0.1138	*0.6978*
Source latitude	0.04105	*0.2769*	-	-	0.08528	*0.0425*	-	-
Stalk length plasticity	-0.2284	*0.2823*	-0.2734	*0.2893*	-0.4327	*0.0373*	-0.2816	*0.239*
Source latitude	0.04282	*0.2611*	-	-	0.07453	*0.0358*	-	-
Spike length plasticity	0.03143	*0.8676*	-0.4314	*0.1912*	-0.2813	*0.1537*	-0.01324	*0.9669*
Source latitude	0.06226	*0.1325*	-	-	0.05621	*0.173*	-	-

Table 5. Effects of plastic traits on reproductive success at MLBS in 2012.

Linear models on the individual effects of reflectance plasticity, onset plasticity, stalk length plasticity, and spike length plasticity by Julian week on cumulative spike and seed production at MLBS in 2012.

MLBS	**Reflectance Plasticity**				**Onset Plasticity**				**Stalk length Plasticity**				**Spike length Plasticity**			
	Spikes		Seeds		Spikes		Seeds		Spikes		Seeds		Spikes		Seeds	
Julian week	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
20	-0.00073	0.9906	0.2143	0.9145	-0.323	0.6609	-1.688	0.943	0.01937	0.9012	0.3278	0.9456	0.4409	0.8316	3.4862	0.9544
22	-0.00073	0.9906	0.2143	0.9145	-0.323	0.6609	-1.688	0.943	0.01937	0.9012	0.3278	0.9456	0.4409	0.8316	3.4862	0.9544
24	-0.00236	0.9699	0.09915	0.9605	-0.3658	0.6194	-2.4844	0.9161	0.04692	0.7638	-0.4841	0.9197	0.6536	0.7526	-15.3194	0.8017
26	-0.01767	0.7777	-0.4062	0.8387	-0.44	0.5502	-9.4229	0.6896	0.04455	0.7754	-2.8017	0.5599	0.2453	0.9058	-73.9289	0.2268
28	-0.03779	0.546	-1.1012	0.5813	-0.2124	0.773	-17.3323	0.4629	0.008501	0.9566	-5.1348	0.2861	0.3212	0.8769	-92.3518	0.1317
30	-0.03404	0.5866	-1.6049	0.4218	-0.2722	0.7116	-21.9293	0.3533	0.0161	0.9178	-5.9007	0.2205	0.6262	0.7626	-112.44	0.067
32	-0.00435	0.9446	-1.1167	0.576	0.3577	0.6271	-11.9096	0.6138	-0.08144	0.6021	-8.2086	0.0891	-0.7283	0.7254	-144.55	0.019
34	0.00703	0.9105	-1.0272	0.6069	0.5316	0.4706	-8.6938	0.7125	-0.08111	0.6036	-8.5028	0.0784	-0.9768	0.6376	-156.96	0.011
36	-0.00383	0.9512	-1.3441	0.501	0.3932	0.5933	-12.7316	0.5896	-0.1103	0.4802	-9.3552	0.0531	-0.9793	0.6368	-157.03	0.011

APPENDIX C

SUPPLEMENTARY FIGURES

Figure C1. Mean stalk length of the first 20 spikes produced.

Mean stalk length of the 34 genotypes that flowered at MLBS and NCA&T in 2011.

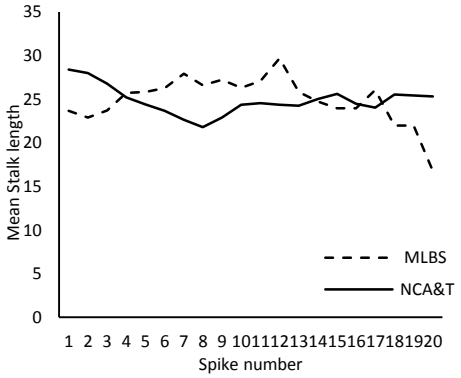


Figure C2. Relationship between spike length and flower number.

Linear relationship between spike length (cm) and flower number from a subsample of 21 spikes from NCA&T and MLBS.

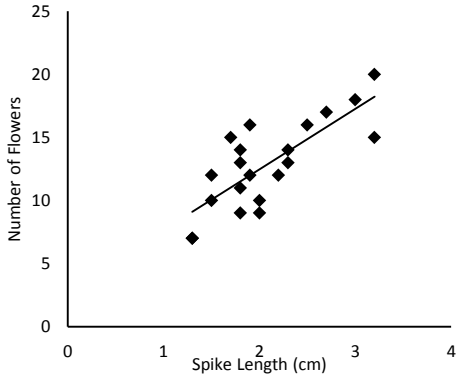


Figure C3. Mean spike length of the first 20 spikes produced.

Mean spike length of the 34 genotypes that flowered at MLBS and NCA&T in 2011.

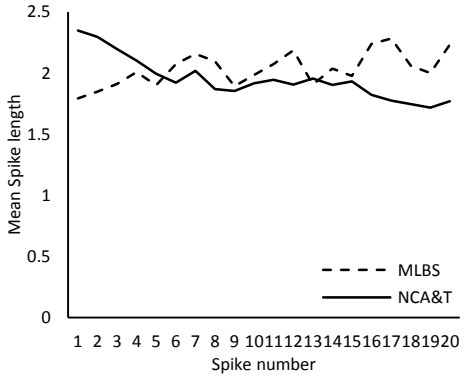


Figure C4. Mean monthly temperatures of low and high latitude genotypes.

Estimated 30-yr mean monthly temperatures during the flowering season for five European population sites (from Lacey et al. 2010). The filled symbols represent high latitude populations, whereas the open symbols represent the low latitude populations.